

TRANSMITTAL

DATE: 10 April 2017

FROM: Mr. Don Smallbeck
Amec Foster Wheeler Project Manager

TO: Ms. Carolyn d'Almeida
USEPA Region 9
75 Hawthorne Street
San Francisco, CA 94105

SUBJECT: Performance-Based Remediation at Former Williams AFB, Arizona
Contract No. FA8903-09-D-8572, Task Order 0002

WE ARE: ☒ enclosing herewith ☐ forwarding under separate cover

Submittal Distribution	Type of Submission	CDRL	Description
AFCEC/CIBW Ms. Catherine Jerrard	1 Hard Copy and 1 Electronic (PDF)	A001	RESPONSE TO ADEQ EVALUATION OF AF RESPONSES TO COMMENTS DATED 25 OCTOBER 2016 ON THE DRAFT FINAL REMEDIAL DESIGN AND REMEDIAL ACTION WORK PLAN ADDENDUM #2 SITE ST012 FORMER WILLIAMS AIR FORCE BASE MESA, ARIZONA
CNTS Mr. Geoff Watkin	1 Hard Copy and 1 Electronic (PDF)		
USEPA Ms. Carolyn d'Almeida Ms. Eva Davis	1 Hard Copy and 1 Electronic (PDF) each		
ADEQ Mr. Wayne Miller	2 Hard Copies and 2 Electronic (PDF)		
UXOPro Mr. Steven Willis	1 Hard Copy (unbound) and 1 Electronic (PDF)		
TechLaw Mr. Michael Anderson	1 Electronic (PDF)		

Remarks:

- | | |
|--|--|
| <input checked="" type="checkbox"/> for review and comment | <input type="checkbox"/> for your files |
| <input type="checkbox"/> reviewed | <input type="checkbox"/> file and reference |
| <input type="checkbox"/> reviewed, see comments, do not resubmit | <input type="checkbox"/> as requested |
| <input type="checkbox"/> not per contract, correct and resubmit | <input type="checkbox"/> reviewed/revised/resubmit |

Comments:

If you have any questions, please contact Don Smallbeck (602) 733-6040 or e-mail don.smallbeck@amecfw.com.



DEPARTMENT OF THE AIR FORCE
AIR FORCE CIVIL ENGINEER CENTER



10 Apr 17

MEMORANDUM FOR Arizona Department of Environmental Quality
Attn: Mr. Wayne Miller, P.E., R.G
1110 West Washington Street, 4415B-1
Phoenix, Arizona 85007

FROM: AFCEC/CIBW
706 Hangar Road
Rome, NY 13441

SUBJECT: Submission of "Response to ADEQ Evaluation of AF Responses to Comments dated 25 October 2016 on the Draft Final Remedial Design and Remedial Action Work Plan Addendum #2, Site ST012, Former Williams Air Force Base, Mesa, Arizona"

1. The Air Force is pleased to submit the attached document, *Response to ADEQ Evaluation of AF Responses to Comments* dated 25 October 2016. ADEQ's evaluation is in response to AF responses to ADEQ and EPA comments (issued 22 August 2016) on the *Draft Final Remedial Design and Remedial Action Work Plan Addendum #2*. ADEQ evaluation comments and AF responses have been included. This Responses to Comments file will be included as an appendix in the Final Remedial Design and Remedial Action Work Plan Addendum #2, Site ST012 when the final report is issued.

2. Please contact me at (315) 356-0810 or catherine.jerrard@us.af.mil if you have any questions regarding this submittal.

CATHERINE JERRARD, PE
BRAC Environmental Coordinator

Attachment:

Response to ADEQ Evaluation of AF Responses to Comments Dated 25 October 2016 on the
Draft Final Remedial Design and Remedial Action Work Plan Addendum #2, Site ST012,
Former Williams Air Force Base, Mesa, Arizona

c: ADEQ – Wayne Miller (2 and 2 CD)
 AFCEC – Catherine Jerrard (1 and 1 CD)
 CNTS – Geoff Watkin (1 and 1 CD)
 TechLaw – Michael Anderson (1 CD)
 USEPA – Carolyn d’Almeida (1 and 1 CD)
 USEPA – Eva Davis (1 and 1 CD)
 UXOPro – Steve Willis (1 and 1 CD)
 File

RESPONSE TO ADEQ EVALUATION COMMENTS OF AF RESPONSES DATED 25 OCTOBER 2016
DRAFT FINAL REMEDIAL DESIGN AND REMEDIAL ACTION WORK PLAN ADDENDUM #2
FORMER LIQUID FUELS STORAGE AREA – SITE ST012
FORMER WILLIAMS AFB, MESA, ARIZONA

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
General Comments					
1	5	Please clarify how chloride concentrations are not expected to inhibit or slow EBR at this site. Chloride levels appear to be extremely high, and may inhibit some sulfate-reducing bacteria as well as others that are hoped to be used for target compound biodegradation during the EBR phase.	It is recognized that chloride can, in general, inhibit cell growth. However, there are no literature or project examples that provide evidence to suggest high concentrations of chloride result in a reduction in effectiveness of sulfate-reducing bacteria. In fact, sulfate-reducing bacteria are common in high salinity marine environments. Based on review of groundwater sample results collected prior to remedial action at ST012, the existing consortia of microorganisms have readily utilized naturally-available TEAs such that the flux of TEAs are rate-limiting in the respiration of the petroleum. The presence of high background chloride levels did not appear to	a. ADEQ reiterates its concern that the current population of sulfate-reducing bacteria is unknown and should be determined prior to the start of EBR. Although there are many populations of sulfate-reducing bacteria that are known to survive and thrive in marine and even hyper-saline environments, these halotolerant communities have special adaptations to allow for this. The Williams AFB location is NOT naturally marine or hyper-saline in nature, and thus the indigenous microbial populations present may not have these special adaptations that would allow for survival in high concentrations of chloride. As a general rule, bacteria not adapted for high-chloride environments will die in	a. The discussion on marine and hypersaline conditions and the references demonstrating salinity effects on SRB are acknowledged. The total dissolved solids (up to 0.4%) and chloride (up to 0.16%) concentrations reported in Addendum 2 for the site do not approach the inhibitory salinity concentrations noted in the Ben-Dov, et al (12 to 16%) and the TDS and chloride concentrations at the site are typically less than the concentrations in the deep spring water reported in Ionescu D., et al where SRB were active. In addition SRBs were detected at ST012 during the EBR field test (see Appendix C of Addendum 2) and their population increased during testing. Addendum 2 includes baseline sampling to evaluate current conditions at the site prior to EBR. SRB populations can be inferred through collection and evaluation of iron, nitrate, and sulfate concentrations and other field parameters. In addition, baseline microbial testing (qPCR for SRB and EBAC) have been added to the proposed program (Table 5-1). Aquifer-borne microbial consortia inclusive of bacteria, archaea, and accessory elements (virus, phage, plasmid, etc.) have shown to be adaptive to changing conditions such as the introduction of inorganic and organic xenobiotic contamination, injections

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
			<p>inhibit biodegradation; instead, biodegradation is likely limited by the availability of TEAs. This discussion will be added to Section 3.1.2.</p>	<p>the presence of high concentrations of the ion. The converse is also generally true: those bacteria adapted to survive in high saline conditions generally cannot survive if introduced to an environment with lower salt concentrations.</p> <p>The response to this comment states that "there are no literature or project examples that provide evidence to suggest high concentrations of chloride result in a reduction in effectiveness of sulfate-reducing bacteria". Dissenting opinions to the response can be found in the following:</p> <ul style="list-style-type: none"> • Oren, A. Bioenergetic Aspects of Halophilism. Microbiol Mol Biol Rev. 1999. 63:334-348. • Ben-Dov, E., et al. Changes in Microbial Diversity in Industrial Wastewater Evaporation Ponds Following Artificial Salination. 2008. FEMS 	<p>and mixing of high-concentration chemical oxidants, and the addition of high-concentration substrates and surfactants. Population abundance and diversity change to the most thermodynamically favorable transfer of electrons from donor to acceptor.</p> <p>Revisions to Section 3.1.2 will include this further discussion concerning inhibition by chloride concentrations.</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
				<p>Microbiology Ecology. 66: 437-446.</p> <ul style="list-style-type: none"> • Lonescu. D., et al. Microbial and Chemical Characterization of Underwater Fresh Water Springs in the Dead Sea. PLOS. 2012. 7:e38319 <p>b. The response states, in part, that "Based on a review of groundwater sample results collected prior to remedial action at ST012, the existing consortia of microorganisms have readily utilized naturally-available TEAs such that the flux of TEAs are rate-limiting in the respiration of the petroleum".</p> <p>Please provide a reference to the specific data and explain how data obtained prior to SEE can show that TEAs are currently limited. Data collected prior to SEE is only applicable to the microbial population as it existed prior to the remedial actions. The status and makeup of the current population is likely</p>	<p>b. A reference to the Treatability Study in Support of Remediation by Natural Attenuation was provided in the Response to ADEQ comments on the Draft Addendum 2. The statement in the previous response, "Based on a review of groundwater sample results collected prior to remedial action at ST012, the existing consortia of microorganisms have readily utilized naturally-available TEAs such that the flux of TEAs are rate-limiting in the respiration of the petroleum" is made to demonstrate that TEA availability and not chloride concentration inhibited SRB prior to SEE. The response is specific to the original comment concerning chloride inhibition. Chloride concentrations are unlikely to be significantly different post-SEE compared to pre-SEE. Therefore, data collected prior to SEE is relevant to the investigating potential observations of chloride inhibition. While the status of the current population is likely different in the area of active SEE, the current population outside the active SEE,</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
				very different from that observed prior to remedial actions.	where the majority of EBR will occur, will have been less influenced by SEE. No additional revisions concerning inhibition by chloride concentrations will be made to the text.
2	6	Please clarify why sulfate should be added to a system that currently has sulfate levels in tested wells as high as 310 mg/L.	Sulfate as high as 310 mg/L are only present upgradient or in areas that do not contain significant COC concentrations. The flux of sulfate by natural groundwater movement through contaminated areas is not sufficient to degrade the remaining mass in the projected timeframe. This discussion will be added to Section 3.1.2	The Air Force RTC states that high sulfate concentrations are only found "upgradient or in areas that do not contain significant COC concentrations." However, a comparison of groundwater data provided in the August 24, 2016 preliminary analytical results table to visual slides presented at the August 24 BCT meeting, shows many of the wells with elevated sulfate concentrations appear to be within the LNAPL extent. Thus, it appears that high sulfate levels are found in areas of significant COC concentrations. Please address and reconcile this issue.	At the time of the previous response, sampling data from Phase I EBR work was not available. Sulfate conditions from the Phase I EBR samples vary by zone and most of the available data is along or outside the perimeter of the SEE TTZs. In the CZ there were some wells with sulfate concentrations lower than typical background concentration but there was no evidence of significant sulfate depletion. In the UWBZ the three highest sulfate concentrations are on the upgradient perimeter of the site. Most of the other UWBZ perimeter wells have lower, but still significant sulfate concentrations and some of the interior UWBZ wells are nearly sulfate depleted (e.g., UWBZ26, UWBZ27, UWBZ33, and UWBZ22). In the LSZ, there were several perimeter wells with concentrations generally consistent with background (e.g., LSZ49, LSZ34, LSZ26, LSZ14, W34, LSZ28), many wells with lower but still significant sulfate concentrations, and some wells with nearly depleted sulfate concentrations (e.g., LSZ37, LSZ50, LSZ38, LSZ39, LSZ11, LSZ48, LSZ29, and W37). It is likely that the sulfate concentrations that are not depleted or partially depleted in areas of LNAPL have been influenced by the groundwater extraction completed during the

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
					<p>post-steam extraction period. Post-steam extraction pulled groundwater with background sulfate from outside the TTZs into areas with LNAPL and sampling may have occurred before the microbial population fully adapted and consumed the sulfate. Although there is variation across the site, areas of depleted sulfate exist and demonstrate evidence of SRB activity at the site and support the goal of EBR to enhance and expand this activity. Current conditions relative to sulfate concentration may have changed since the samples were collected in July 2016. Due to the delay in EBR implementation, resampling is planned prior to injection to re-establish the baseline conditions. Current site conditions will be evaluated to determine if modifications are needed to the injection plan.</p> <p>No revisions concerning the need for sulfate injections will be made to the text.</p>
Specific Comments					
3	2	Please clarify the statement that, "sulfate amendment can either be used solely or in combination with aerobic methods to achieve remediation goals." The use of sulfate to stimulate the strongly anaerobic process of sulfate-reduction is not compatible with aerobic	The different TEAs could be implemented sequentially or in different areas. The sentence was revised as follows: "Sulfate amendment can either be used solely or in combination with aerobic methods (either sequentially or in different areas) to achieve remediation goals."	Please explain how an aerobic method will be successfully used "sequentially" with a strongly anaerobic method such as sulfate-reduction. Please provide a peer-reviewed reference for such a "sequential" use of widely differing bioremediation methods for in-situ remediation of hydrocarbons.	While the use of aerobic methods cannot be totally ruled out for future remediation in specific areas, the current plan is to initially focus on anaerobic sulfate-reduction methods. The originally referenced statement will be deleted and the revisions described in the previous response will not be made.

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
		methods of bioremediation. Sulfate reduction occurs only under highly reduced environmental conditions, while aerobic respiration occurs only under highly oxidized environmental conditions. Thus, sulfate-reduction cannot be used in combination with aerobic methods.			
4	4	See the evaluation of the response to ADEQ General Comment 1 (original comment date February 11, 2016). The statement assumes a prior knowledge that does not appear to exist regarding the indigenous microbial population. Furthermore, this statement assumes that sulfate-reducers dominate the indigenous population - something that has not been proven. ADEQ has specifically questioned and asked to have this investigated.	The point of the bullet is that the sulfate reducing bacteria stimulated by the EBR will also have a long-term source of sulfate from upgradient groundwater. With implementation of EBR, sulfate reducing bacteria will be the dominant established population. The dominant established population will be confirmed via microbial analysis between six and twelve months following the initiation of sulfate injections, as shown in Table 5-1. The bullet has been revised as follows to clarify: "influent upgradient background	a. The condition of sulfate-reducers dominating the current, indigenous microbial population has not been proven, and ADEQ requests that this be investigated. b. Please explain how the AF plans to confirm changes from the "established" microbial and chemical conditions if current,	a. Microbial testing (qPCR for SRB and EBAC) has been added to baseline sampling for six wells (two in each zone) in Table 5-1, however, the objective of this testing is to establish a baseline for comparison and not to establish that SRB are currently dominating the microbial population. SRB have been observed to be present and active at the site (see Appendix C of Addendum 2); however, under sulfate-limited conditions they would not be expected to be the current dominant population in locations of abundant substrate (LNAPL). The EBR monitoring program includes analysis to support the assessment for establishing that SRB are a dominant population once active EBR is implemented. b. The objective is to show that the desired conditions are established for EBR following injections. "Established" in the initial AF response is in reference to microbial conditions and population following sulfate

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
			sulfate can supplement sulfate amendments to promote petroleum hydrocarbon degradation during and after EBR without having to change the established bacterial populations or redox conditions;"	"established" microbial populations and chemical conditions are not known prior to EBR inception. ADEQ suggests performing baseline microbial analyses in addition to geochemical sampling to establish the current site conditions. This would allow for proper and meaningful comparisons between the current conditions and those during and after EBR.	injections. Microbial testing (qPCR for SRB and EBAC) has been added to baseline sampling for six wells (two in each zone) in Table 5-1. No revisions will be made to the text except for inclusion of the baseline qPCR samples in Table 5-1.
5	5	What specific "rate-limiting geochemical conditions" will be monitored, and what is the plan for maintaining effective EBR if one of these adverse conditions is encountered?	Changed text in Section 3.2.3: "...or rate-limiting geochemical conditions (e.g., pH, oxidation-reduction potential (ORP), nitrogen and micronutrient concentration)." If EBR is shown to be affected by monitored rate-limiting geochemical conditions, additional amendments may be added to the subsurface using the on-site injection system. A discussion of this situation is included in Section 4.2.3: Micronutrient Dosing.	Please provide all data collected during sampling (i.e., all field parameters, water level measurements, sample depth, etc.) when transmitting preliminary analytical data.	In the future, field parameters will be added to the data tables, including preliminary tables. Sample depth is currently provided in the data tables. No changes will be made to the text.

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
6	7	Please detail how both population surge/crash and plugging of the formation with biomass will be prevented.	<p>Biomass is expected to surge in the formation where sulfate concentrations are optimum and above twice half saturation. In these locations some level of formation plugging or reduction of pore space is inevitable, however; it is anticipated to have minimal negative consequences on the remediation of petroleum hydrocarbons. Conversely, the population surge will assist in retaining TEA in the vicinity of petroleum impacted media.</p> <p>Microbial populations are expected to follow typical growth phases with the introduction of abundant TEA. The immediate response is generally a lag phase (little or no population growth) during which the microorganisms adjust or evolve to the change in geochemical conditions. As the consortium diversity realigns,</p>	<p>a. Please clarify the phrase "twice half saturation." Does the AF propose injecting full saturation concentrations of sulfate, with expectations that this sulfate will actually travel through the formation? At full saturation concentrations, sulfate will precipitate out of solution.</p> <p>b. Please explain how the anticipated plugging of formation pore spaces will "have minimal negative consequences on the remediation of petroleum hydrocarbons" when a wealth of published data specifically cites this issue as a strong and negative impact on overall mass reduction at sites. Multiple EPA guidance documents specifically cite plugging of a formation as a negative factor to avoid when trying to stimulate biodegradation. Please explain why it won't negatively impact hydrocarbon degradation</p>	<p>a. The phrase "twice half saturation" is in reference to microbial growth kinetics not the solubility of sulfate. In Monod kinetic model half-saturation is the concentration at which the growth rate is ½ of the maximum growth rate.</p> <p>Injections will be at or above concentrations required to achieve maximum growth rate but will be below full solubility concentrations.</p> <p>No changes will be made to the text</p> <p>b. If plugging of the formation occurs it is not a positive development or desired outcome; however, unlike remedial technologies that rely on: 1) a constant feed of TEA to the impacted media (e.g., in-situ air sparging); or 2) contact of a substrate amendment with the contaminant (e.g., enhanced reductive dechlorination), the EBR process described relies on a high-concentration dosing of sulfate to reach LNAPL impacted media. It is likely that if biofouling occurs in the aquifer pore-spaces then it is occurring because at that location significant sulfate has been utilized in the presence of an abundance of substrate. Based on the current understanding of the ST012 aquifer, the only abundant carbon source (substrate) significant enough to potentially clog pores is LNAPL. This would be an indication that significant petroleum degradation is occurring (i.e., a positive development</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
			<p>exponential growth is anticipated until zero-order or maximum utilization is reached. Since the petroleum substrate is expected to change in bioavailability over time, variability in the maximum utilization rate and consortium diversity is also anticipated to change. Ultimately, the system is expected to return to natural or background levels and diversity as the petroleum hydrocarbon source and sulfate are degraded and mineralized.</p> <p>The following text was added to Section 4.2.5: "Biofouling. It is anticipated that the high ionic strength of the injection solution will reduce plugging of the formation with biomass by inhibiting microbial growth in the immediate vicinity of injection wells, thereby allowing use of these wells for future dosing. However, it is also anticipated that as</p>	<p>at this location, when it is so strongly avoided in other locations.</p> <p>c. Please explain how "the (resulting microbial) surge will assist in retaining TEA in the vicinity of the petroleum impacted media".</p> <p>d. The AF response references "typical growth phases." Please explain how these microbial growth phases will be monitored during EBR. These growth curves are in response to total nutrient availability and not just a single element such as a terminal electron acceptor.</p>	<p>despite the clogging). As indicated in previous responses to ADEQ comments on biofouling, the work plan contains methods for managing the biofouling so that it will not have a significant impact on the overall effectiveness of EBR.</p> <p>c. The microbial surge will consume much of the TEA locally based on the amount of hydrocarbon present, thus initially allowing less TEA to move beyond the area of significant TPH presence. As the TPH is reduced more TEA is available downgradient for consumption and concomitant hydrocarbon reduction.</p> <p>No changes will be made to the text.</p> <p>d. The term "typical growth phases" is a general term used to denote overall microbial growth as a function of hydrocarbon utilization. It is expected that several simultaneous processes will occur during the lag and exponential growth phases before zero-order sulfate utilization is realized. These processes include population abundance and diversity shifts in response to the sulfate; generation of biological surfactants and films; and initial diffusion/dispersion of the sulfate following injection. Although it is a possibility that instead of petroleum-hydrocarbon substrate, some other factor such as the availability of key- or micro-nutrient could be the rate limiting factor, previous site data indicates that sulfate availability is the</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
			<p>sulfate concentrations drop at the injection well sites microbial blooms may occur along with biofouling of the well screen and filter pack. If the wells are affected by biofouling, one or more of the following two courses of action (or similar variations on these actions) will be implemented:</p> <ol style="list-style-type: none"> 1. Injection wells will be pressurized to deliver TEA solutions into wells. 2. Injection and/or extraction wells will be redeveloped by mechanical removal (e.g., hydrojet, surge, bail) and/or chemical addition (e.g., biocide) could be employed to restore well function. " 	<p>e. The response states that the addition of a biocide is a possible remedy to biofouling.</p> <p>f. However, the addition of a biocide may kill the very microbes needed for EBR to work. Please explain how poisoning of the hydrocarbon-degrading population will be avoided if biocide use is to occur. Include in this explanation the details of how the health of the hydrocarbon-degrading population will be confirmed during and after biocide use.</p>	<p>primary rate limiting factor affecting petroleum hydrocarbon degradation. The measurement of constant sulfate utilization will be evidence of zero-order utilization for hydrocarbon degradation. Therefore, sulfate concentration trends will be the primary feedback used to assess the establishment of microbial growth at zero-order sulfate utilization. In addition to sulfate, pH, eH (or redox), and sulfide will be monitored to support the assessment of microbial growth and/or determine if some other factor besides substrate (petroleum hydrocarbons) is rate-limiting.</p> <p>No changes will be made to the text.</p> <p>e. Acknowledged.</p> <p>No changes will be made to the text.</p> <p>f. The response was to indicate that a possible remedy for biofouling is the use of a biocide, however, as indicated several other techniques are available to manage biofouling without resorting to biocide use. If a biocide addition is necessary a field variance will be prepared providing site specific details based on performance monitoring of the EBR remedy. Addendum 2 will be revised accordingly.</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
7	Item 3	3a) Please detail how the proper length of time for sampler deployment will be determined and followed. The response states that the Bio-trap® SIP sampler will be deployed for approximately one month before being retrieved for analysis. However, this is a general timeframe provided by Microbial Insights to be used as a starting point in determining the proper length of deployment time. This time length should be adjusted based on site geochemical conditions and target compounds. If the assumed sulfate-reducing conditions are dominant, then experience with these samplers in anaerobic environments suggests that one month may not be enough time to properly allow for adequate target compound	3a) The timing for deployment of Bio-traps for stable isotope probing (SIP) following the addition of sulfate will be based on feedback from the groundwater sampling. Sulfate, COC concentrations, and general water quality sample results will be used to assess the timing and final location for deployment of the post-sulfate addition SIP. It is important that the SIP be deployed after the lag-phase and preferably after the exponential growth-phase has occurred. Depending on the feedback from the groundwater analyses SIP may be deployed at more than one time step. Additionally, the duration of the deployment will be adjusted based on feedback; however, the one-month, rule-of-thumb will likely prevail as a reasonable timeframe for attachment and generation of at least some biofilm. The	The AF response references specific microbial growth stages. In particular, the AF response states that "it is important that the SIP be deployed after the lag-phase and preferably after the exponential growth-phase has occurred." a. As only geochemical testing is referenced, will this time point be determined from a microbial standpoint?	a. Geochemical testing, primarily trends in eH (redox potential), pH, sulfate, and sulfide, is an adequate indirect measurement and will be assessed to determine the appropriate timing for deployment of the SIP bio-trap sampler. Stability or a trend towards stability in eH and pH and a constant sulfate utilization rate is desired to determine that sulfate is being utilized zero-order. To address the potential that a longer deployment is necessary, in two of the six wells selected for SIP analysis two extra SIP bio-trap samplers will be deployed. One well with extra bio-trap samplers will be inside and one outside the former TTZ. Following one month of in-well incubation one bio-trap from all six wells will be removed and submitted for analysis. If SIP analyses on the six traps analyzed following one month of in-well incubation are negative or inconclusive, the additional traps will be used to assess if longer in-well incubation is needed. One of the extra bio-trap samplers will be pulled for the two wells approximately

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
		mineralization or conversion to biomass.	substrate utilization rates at zero-order are anticipated to be significantly higher than ambient biodegradation. At these higher rates reattachment and growth on the Bio-trap media is anticipated to be faster post-sulfate addition.	b. If geochemical parameters are being correlated to, and will be used to determine in situ growth stages, please provide a peer-reviewed reference for this protocol for contaminated sites.	<p>two months following their placement. If these bio-trap's results are negative or inconclusive then the last biotrap samplers will be retrieved and submitted for analysis following approximately three months of in-well incubation. Depending on the results and following the completion of this first round of SIP analysis an additional round of SIP bio-trap sampling and analysis will be conducted on select remaining wells, if necessary.</p> <p>The text will be updated to describe the extra bio-traps.</p> <p>b. Microorganisms have the ability to degrade many organic compounds, including petroleum hydrocarbons present in the subsurface. The most common electron acceptors for oxidation include O₂, Mn⁴⁺, NO₃⁻, Fe³⁺, and SO₄²⁻ (Chapelle 1993). Research has shown that the majority of petroleum hydrocarbons will biodegrade using oxygen as the terminal electron acceptor (Borden et al. 1995); however, subsurface systems are often anaerobic due to the low aqueous solubility of oxygen and rapid consumption of oxygen by subsurface microorganisms (Wiedemeier et al. 1999). This is certainly the case, and has been for some time (decades) at ST012. Fortunately, alternative electron acceptors (Mn⁴⁺, NO₃⁻, Fe³⁺, and SO₄²⁻) are often available within the subsurface (Chapelle 1993). Petroleum hydrocarbon degradation has been shown to</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
					<p>occur under anaerobic conditions, including via the reduction of sulfate (Rueter et al. 1994; Lovley and Phillips 1994), nitrate, or ferric iron. Once these acceptors have been exhausted, further depletion of hydrocarbon can occur via reduction of organic carbon to methane (CH₄) (Chapelle 1999). This process is referred to as methanogenesis.</p> <p>Sulfate-reducing conditions are often observed in subsurface environments containing LNAPL. To date, the majority of sulfate-reducing bacteria isolated from environments containing petroleum hydrocarbons are associated with the family Desulfobacteraceae. Several mesophilic alkane-degrading sulfate-reducers have been isolated including Desulfococcus oleovorans Hxd3 from an oil-water separator (Aeckersberg et al. 1991), strain Pnd3 from petroleum-contaminated marine sediments (Aeckersberg et al. 1998), Desulfatibacillum alkenivorans AK-01 from petroleum-contaminated estuarine sediments and Desulfatibacillum aliphaticivorans CV2803 from hydrocarbon polluted marine sediments (Cravo-Laureau et al. 2004). A thermophilic alkane-degrading sulfate-reducer, Desulfothermus naphthae TD3 (member of genus Desulfotomaculum), capable of oxidizing n-alkanes at 60°C was isolated from Guaymas basin (a site in the Gulf of California which has hydrothermal activity) (Rueter et al. 1994). In addition to bacteria, archaeal sulfate reducers were identified in LNAPL impacted soil (Benlloch et al. 2002).</p>

Evaluation Item	Comment #	ADEQ Original Comment	AF Response to ADEQ Original Comment	ADEQ Evaluation of AF Response	AF Response to ADEQ Evaluation
					<p>Much in the same way that geochemical indicators are used to monitor intrinsic bioremediation of petroleum hydrocarbons (Borden, 1995, ITRC, 2009, Wiedemeier et al, 1999), they can be used to assess enhanced bioremediation of petroleum-hydrocarbons in the subsurface. Comparison of background (or initial amended) terminal electron acceptor concentrations with concentrations in the LNAPL impacted area over time provides information on utilization rate. Acceleration and deceleration of sulfate utilization will be key indicators of lag and exponential growth phases. In addition, eH and pH will be used to assess the depletion of substrate petroleum hydrocarbons. Trends showing eH and pH decline can be strong indicators of exponential and stable growth phases.</p> <p>No changes will be made to the text.</p>

References

- Aeckersberg F, Bak F, Widdel F (1991) Anaerobic oxidation of saturated hydrocarbons to CO₂ by a new type of sulfate-reducing bacterium. Archives of microbiology, 156, pp.5–14.
- Aeckersberg F, Rainey F, Widdel, F (1998) Growth, natural relationships, cellular fatty acids and metabolic adaptation of sulfate-reducing bacteria that utilize long-chain alkanes under anoxic conditions. Archives of microbiology, 170(5), pp.361–9.
- Benlloch S, López-López A, Casamayor E, Øvreås L, Goddard V, Daae F, Smerdon G, Massana R, Joint I, Thingstad F, Pedrós-Alió C, Rodríguez-Valera F (2002) Prokaryotic genetic diversity throughout the salinity gradient of a coastal solar saltern. Environmental microbiology, 4(6), pp.349–60.
- Borden RC, Gomez CA, Becker MT (1995) Geochemical Indicators of Intrinsic Bioremediation. Ground Water, 33(2), pp.180–189.

- Chapelle FH (1999) Bioremediation of Petroleum Hydrocarbon-Contaminated Ground Water: The Perspectives of History and Hydrology. *Ground Water*, 37(1), pp.122–132.
- Chapelle FH (1993) *Ground-water Microbiology and Geochemistry* 2nd ed., New York, NY: John Willey & Sons, Inc.
- Cravo-Laureau, Cristiana et al., 2004. *Desulfatibacillum alkenivorans* sp. nov., a novel n-alkene-degrading, sulfate-reducing bacterium, and emended description of the genus *Desulfatibacillum*. *International journal of systematic and evolutionary microbiology*, 54(Pt 5), pp.1639–42.
- ITRC 2009 Evaluating Natural Source Zone Depletion at Sites with LNAPL, LNAPL-1, Technology Overview, Interstate Technology & Regulatory Council, Washington D.C., 76 pp.
- Lovley, Derek R & Phillips, E.J.P., 1994. Novel Processes for Anaerobic Sulfate Production from Elemental Sulfur by Novel Processes for Anaerobic Sulfate Production from Elemental Sulfur by Sulfate-Reducing Bacteria. , 60(7).
- Rueter, P. et al., 1994. Anaerobic oxidation of hydrocarbons in crude oil by new types of sulfate-reducing bacteria. *Nature*, 372, pp.455–458.
- Wiedemeier, T.H., Wilson, J. T., Kampbell, D.H., Miller, R.N., and Hansen, J.E., Technical Protocol for Implementing Intrinsic Remediation with Long-Term Monitoring for Natural Attenuation of Fuel Contamination Dissolved in Groundwater. Prepared for Air Force Center for Environmental Excellence. Revision 0. March 1999.
- Wiedemeier, E.T., Raifai, H.S. & Newell, C.J., 1999. *Natural Attenuation of Fuels and Chlorinated solvents in Subsurface*, John Willey & Sons, Inc.